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Indian Standard

METHODS OF SAMPLING AND TEST FOR FERTILIZERS

PART 5 DETERMINATION OF SECONDARY ELEMENTS AND MICRONUTRIENTS

(First Revision)

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NEW DELHI 110002

Gr 9 *February* 1986

Indian Standard

METHODS OF SAMPLING AND TEST FOR FERTILIZERS

PART 5 DETERMINATION OF SECONDARY ELEMENTS AND MICRONUTRIENTS

(First Revision)

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Indian Standard

METHODS OF SAMPLING AND TEST FOR FERTILIZERS

PART 5 DETERMINATION OF SECONDARY ELEMENTS AND MICRONUTRIENTS

(First Revision)

0. FOREWORD

0.1 This Indian Standard (First Revision) was adopted by the Indian Standards Institution on 6 March 1985, after the draft finalized by the Fertilizers Sectional Committee had been approved by the Petroleum, Coal and Related Products Division Council.

0.2 This standard was first published in 1971. Taking into consideration the views of the manufacturers, testing authorities and the technologists, the Sectional Committee decided to revise this standard and felt that the methods of test should be related to those generally used in the industrial laboratories.

0.3 In the present revision 'Atomic absorption spectrophotometric method' has been included. The methods of test for chromium, nickel and fluorine have been excluded from this standard and the same have been included in IS : 6092 (Part 6)-1985* as these elements are considered as impurities and not as micronutrients. The title of the standard has been modified accordingly.

0.4 Cross-reference of corresponding method of test between IS : 6092 (Part 5)-1971 and IS : 6092 (Part 5)-1985 (*first revision*) has been given in Appendix A.

0.5 In the preparation of this standard, consideration has been given to the need for maintaining co-ordination with the methods for sampling and test for fertilizers prescribed under the Fertilizer (Control) Order, 1957 and the Essential Commodities Act, 1955 (incorporating amendment up to August 1981), of the Government of India. However, this standard is subject to the provision imposed under this order wherever applicable.

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*Methods of sampling and test for fertilizers: Part 6 Determination of moisture and impurities (*first revision*).

0.6 In the preparation of this standard considerable assistance has been derived from the following:

Official methods of analysis of the Association of Official Analytical Chemist. Washington DC, USA, 1975.

Recommended analytical methods of the National Plant Food Institute, Washington DC, USA.

Determination of trace elements, with special reference to fertilizers and feeding stuffs. Society for Analytical Chemistry, UK. 1963. W. Heffer & Sons Ltd, Cambridge.

Fertilizer sampling and analytical methods. The Fertilizer Institute Product Quality Committee, Washington DC, USA, 1979.

0.7 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard (Part 5) prescribes the methods for determination of secondary nutrients (calcium, magnesium and sulphur) and micronutrients (iron, zinc, manganese, copper, boron, molybdenum, cobalt, lead and chlorine) in fertilizers, including fertilizer mixtures.

2. SAMPLING

2.1 Representative test samples of the fertilizer shall be drawn as prescribed in IS : 6092 (Part 1)-1985†.

3. QUALITY OF REAGENTS AND GLASSWARES

3.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1977‡) shall be employed in tests.

NOTE — ' Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis

3.2 For micronutrients work, all glasswares must be of resistant neutral glass except for boron for which soda glass or specially made ' boron free' glasswares are to be used. Good quality heat resistant polyethylene wares are quite suitable except when subjected to high temperature.

4. PREPARATION OF SAMPLE SOLUTION

4.0 It is a general method, specific procedures have been given under individual elements.

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*Rules for rounding off numerical values (*revised*).

†Methods of sampling and test for fertilizers : Part 1 Sampling.

‡Specification for water for general laboratory use (*second revision*).

4.1 For Organic Fertilizers — Weigh a suitable quantity (usually about 5 g) of the dry sample in a silica basin (*see* Note 2), cover with a silica clock glass, and place in a cool silica-lined muffle furnace. Raise the temperature step by step to about 450°C (*see* Note 3), and allow the ashing to proceed over-night; a slow movement of air through the furnace during the initial stages of ashing is desirable. When all the organic matter has been destroyed, cool, add 10 ml of dilute hydrochloric acid (1 + 1), and evaporate to dryness on a water-bath. Extract the soluble salts from the residue with two successive 10 ml portions of boiling dilute hydrochloric acid (1 + 5), decanting the solution each time through the same filter paper (Whatman No. 40 or equivalent) into a 100 ml calibrated flask. Then add 5 ml of dilute hydrochloric acid (1 + 1) and about 5 ml of dilute nitric acid (1 + 2) to the residue in the basin, and evaporate the mixture to dryness on a hot-plate at low heat so as to remove all the nitric acid.F inally, add a further 10 ml of boiling dilute hydrochloric acid (1 + 5) to the residue and filter the solution through the same paper into the flask. Dilute the combined extracts to the mark with water, washing the filter paper in the process. This final solution should be approximately normal with respect to hydrochloric acid.

NOTE 1 — This type of dry ashing is not applicable for sulphur and chlorine which are likely to be partly lost.

NOTE 2 —Platinum basins are preferable for ashing, but shall not be used for the subsequent evaporation to dryness with nitric acid.

NOTE 3 — It is recommended that the ashing be conducted as low a temperature as possible. In the case of organic matter rich materials, care shall be taken to avoid ignition of the sample. These should preferably be carefully carbonised first over a low heat (200°C or less) before placing the dish inside the furnace.

4.1.1 *Preparation of Sample Solution for Estimation of Sulphur* — To the weighed sample (5 g) in a silica basin, 5 ml of 20 percent solution of magnesium nitrate (sulphur free) is mixed thoroughly and carefully dried on steam bath and then on hot plate taking care against any spurting. Then the contents are carefully carbonised at low heat before igniting in the muffle furnace.

4.1.2 *Preparation of Sample Solution for Estimation of Chlorine* — Weigh 5 g of the sample in a silica basin. Mix the test sample intimately with one-fifth of its weight (1 g in this case) with pure calcium oxide, prepared by igniting reagent grade calcium carbonate at 750-800°C to a constant weight. Moisten to a slurry with distilled water and mix again. Dry the basin thoroughly and ignite the material as in **4.1.1**.

NOTE — For boron estimation, ignition with calcium hydroxide is done (*see* **6.1.1.4**).

4.2 For Inorganic Fertilizers — Boil the sample (usually about 5 g) in 20 ml of hydrochloric acid (1 + 1) for 30 minutes and filter through

Whatman No. 40 filter paper. Follow with repeated extraction with boiling dilute hydrochloric acid (1 + 5) in a manner similar to that described above for the ash of organic fertilizers (**4.1**) but with some more acid that may be required to effect solution. The extract should be made up to 100 ml.

Note — Even the best quality of reagents available are likely to contain traces of some of these elements and therefore a blank without sample must be run along with.

5. METHODS OF TEST FOR SECONDARY ELEMENTS

5.1 Determination of Calcium

5.1.1 *Procedure*

5.1.1.1 Transfer 10 ml aliquot of the sample solution (*see* **4**) to a 250 ml beaker and dilute to 100 ml. Add 2 drops bromophenol blue indicator. Add ammonium hydroxide (1 + 4) until indicates changes from yellow to green (not blue). If overrun, bring back with hydrochloric acid (1 + 4). This gives *p*H of 3·5 to 4·0 . Dilute to 150 ml, bring to boiling point and add 30 ml of saturated hot ammonium oxalate [$(NH_4)_2C_2O_4$] solution slowly, stirring continuously. If colour changes from green to blue or yellow again, adjust to green. Digest on water bath for 1 hour, or let it stand over night, and cool to room temperature.

Note 1 — For materials rich in calcium, the sample solution should be further diluted 5 times (20 to 100 ml) from which 10 to 20 ml aliquot should be taken for precipitation of calcium.

Note 2 — If appreciable quantities of Fe/Al are present as seen from their hydroxides precipitated on adding ammonium hydroxide, these should be filtered off.

Note 3 — If appreciable amount of P_2O_5 is present, it is always advisable to remove iron and aluminium. This may be done as under :

To a suitable aliquot depending on the calcium content, add 2 drops of bromophenol blue indicator and neutralize with ammonia till solution is blue. Just acidify with drop by drop addition of 1 : 10 HCl. Add 20 ml of ammonium acetate solution (5 ml acetic acid and 15 ml of saturated solution of ammonium acetate). Heat to boil. The phosphates of iron and aluminium get precipitated which can be removed by filtration. If the calcium content is high, there is a possibility of co-precipitation of calcium phosphate. This precipitate can be redissolved in hot 1 : 10 HCl, and reprecipitated as above. This eliminates loss of calcium alongwith iron and aluminium phosphate. Both the filtrates can be combined and the calcium precipitated as oxalate using ammonium acetate.

5.1.1.2 Filter supernatant liquid quantitatively through filter paper (Whatman No. 42) or Gooch or fitted glass filter crucible and wash precipitate thoroughly with ammonium hydroxide (1 + 50), finally with water. Wash down with water, the precipitate in original beaker and add mixture of 125 ml water and 5 ml of sulphuric acid. Heat to about 70°C and titrate with 0·1 N potassium permanganate until first slight pink colour appears. Transfer the filter paper or the pad and repeat the titration at about 70°C till pink colour end point appears which persists for 15 seconds.

5.1.1.3 Correct for blank and calculate the calcium.

5.2 Determination of Magnesium

5.2.0 *General* — Two methods are given below of which one may be selected depending upon the quantity of magnesium estimated.

5.2.1 *Spectrophotometric Procedure* — For magnesium contents up to 100 micrograms in the final aliquot.

5.2.1.1 In this method, after destruction of organic matter and removal of sesquioxides matter, if necessary, the magnesium in hydrochloric acid solution is complexed with titan yellow, and the excess of dye is extracted from the reaction mixture and determined absorptiometrically.

5.2.2 *Reagents*

5.2.2.1 *Dilute hydrochloric acid* — 1 : 9 (*v*/*v*).

5.2.2.2 *Calcium compensating reagent* — Dissolve 0·5 g of calcium carbonate analytical reagent grade in 20 to 25 ml of dilute hydrochloric acid and make up to 1 000 ml.

5.2.2.3 *Oxalic acid solution* — Dissolve in 100 ml of water 1·5 g of crystalline oxalic acid [$(CO_2H)_2 \cdot 2H_2O$].

5.2.2.4 *Sodium hydroxide solution* — 10 percent approximately.

5.2.2.5 *Tartrate reagent* — Dissolve, separately, 10 g of sodium hydrogen tartrate, 2·5 g of hydrazine sulphate, and 10 g of mannitol in water, mix the three solutions and dilute to 1 litre with water.

5.2.2.6 *Titan yellow solution* — Dissolve 0·03 g of titan yellow in 100 ml of water and add 1 drop of sodium hydroxide solution.

NOTE — The quality of the dye is important as inferior materials give limited optical density ranges. Each new batch of titan yellow must be tested rigorously for suitability.

5.2.2.7 *Dye solvent* — Mix 3 parts of *n*-butanol with 2 parts (*v*/*v*) of absolute alcohol (ethanol).

5.2.2.8 *Acetone* — *see* IS : 170-1976*.

5.2.2.9 *Standard magnesium solution* — Dissolve 1·013 6 g of crystalline magnesium sulphate ($MgSO_4 . 7H_2O$) in water, and dilute to 250 ml (with

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*Specification for acetone (*second revision*).

water). One millilitre of the solution contains 400 micrograms of magnesium.

5.2.3 *Procedure*

5.2.3.1 Add to a suitable aliquot of the acid solution prepared in **4.1** or **4.2**, neccessary amount of dilute hydrochloric acid so that each millilitre contains not more than 20 micrograms of magnesium. To 5·0 ml of this diluted solution, add 5 ml of calcium compensating reagent (*see* Note 1), 5 ml of oxalic acid solution and sufficient sodium hydroxide solution to bring the *p*H to between 4 and 5 (usually about 3 to 5 ml may be necessary), mix well, and keep aside for 1 hour. Run down 5 ml of tartrate reagent, followed by 2·5 ml of titan yellow solution, mix well by vigorous agitation, make alkaline with 20 ml of sodium hydroxide solution and allow to stand overnight. Extract the excess dye by shaking the mixture vigorously with 50 ml of dye solvent, allow to stand for 30 minutes, filter the alcoholic layer into a small conical flask containing 0·5 ml of acetone, and mix.

NOTE 1 — If finally, the calcium content of the mixture exceeds 1·5 mg, slightly high results may be obtained. If the quantity of calcium present in the test aliquot is known, the amount of compensating reagent to de added may be adjusted to give a constant calcium content of 1 mg.

NOTE 2 — A blank determination on all reagents used should be carried out.

5.2.3.2 Measure within 30 minutes the optical density of the test solution in a spectrophotometer at a wavelength of 495 nm using the blank solution in the comparison cell. Read the number of micrograms of magnesium equivalent to the observed optical density from a previously prepared standard curve and accordingly find out the magnesium content in the sample.

Prepare the calibration graph covering the range 0 to 100 micrograms of magnesium by drawing out aliquots of a suitable dilution of the standard magnesium solution through the whole procedure described above. Measure the optical densities and plot against the number of micrograms of magnesium. As a check, one or two standards should also be taken each time the test samples are assayed.

5.2.4 *EDTA Titration Method*

5.2.4.0 This method is applicable to samples containing not more than 0·25 percent manganese or zinc.

5.2.4.1 *Reagents*

a) *Buffer Solution* — *p*H 10. Dissolve 67·5 g of ammonium chloride in 200 ml of water and 570 ml ammonium hydroxide and dilute to 1 litre.

b) *Eriochrome Black T Indicator* — Dissolve 0·1 g of eriochrome black T in 25 ml of methyl alcohol.

c) *Standard Magnesium Solution* — Weigh 2·464 0 g of magnesium sulphate ($MgSO_4 \cdot 7H_2O$) and dissolve it in water. Make up the volume to one litre with double distilled water.

d) *Ethylenediamine Tetra-Acetate (EDTA) Solution* — Dissolve 3·72 g of disodium ethylenediamine tetra-acetate dihydrate in water and make up the volume to one litre.

5.2.4.2 *Standardization* — Pipette 10 ml of standard magnesium sulphate solution in a conical flask. Add 20 ml of water, one millilitre of eriochrome black T indicator and 25 ml of ammonium hydroxide — ammonium chloride buffer solution. Heat at 40 to 50°C and titrate with EDTA solution maintaining the temperature between 40 to 50°C until the colour changes from winered to distinct blue.

5.2.4.3 *Calculation*

$$\text{Molarity of EDTA solution} = 10\frac{M_1}{V_1}$$

where

M_1 = Molarity of standard magnesium solution; and

V_1 = volume in ml of EDTA solution used for titration.

5.2.4.4 *Preparation of solution* — Weigh 5 g of sample accurately dissolve in distilled water and add 1 ml of sulphuric acid. Filter the solution and make up to 250 ml with water in a volumetric flask. Take 50 ml of the above solution in a beaker, heat, pass hydrogen sulphide gas and ensure complete precipitation. Filter hot. To the filtrate, add few drops of concentrated nitric acid and boil for 10 minutes. Cool, add solid ammonium chloride (about 2 grams), boil and cool, add ammonium hydroxide till the strong smell of ammonia comes and filter the precipitate through sintered crucible, take this filtrate and add dilute sulphuric acid till the solution is acidic (test with methyl red), heat the solution to boiling and add excess of diammonium hydrogen phosphate with continuous stirring. Add 10 percent ammonia solution with continuous stirring till the solution is just alkaline (test with methyl red) white precipitate of zinc ammonium phosphate will be formed (the optimum *p*H for precipitation is 6-7), allow it to stand for 3 to 4 hours. Filter through Whatman filter paper No. 40. Collect the filtrate in a volumetric flask. Make up the volume say (100 ml). Take a suitable aliquot (10 ml) for the determination of magnesium. Add 20 ml of water, one ml of eriochrome black-T indicator and 20 ml of ammonium hydroxide ammonium chloride buffer solution.

Heat to 40 to 50°C and titrate with standard EDTA solution, maintaining the temperature between 40 and 50°C until the colour changes from wine red to distinct blue.

5.2.4.5 *Calculation*

$$1 \text{ ml of } 0{\cdot}01 \text{ M EDTA} = 0{\cdot}243\ 2 \text{ mg of 'Mg'}$$

$$\text{Magnesium, percent by mass} = \frac{V \times 0{\cdot}243\ 2}{5}$$

where

V = volume of 0·1 M EDTA used for titration.

5.3 Determination of Sulphur

5.3.0 *General* — Three methods for determination of sulphur are given below. Gravimetric barium sulphate method shall be used in case of any dispute.

5.3.1 *Total Sulphur*

5.3.1.1 *Reagent*

a) *Barium chloride solution* — 10 percent. Dissolve 100 g barium chloride $BaCl_2{\cdot}2H_2O$ in 900 ml of water and filter through Whatman No. 42 paper, or equivalent. One ml of this solution is equal to 14 mg of sulphur.

b) *Bromine in carbon tetrachloride* — 10 percent. Add 10 g of bromine to 90 g carbon tetrachloride (reagent grade). Stir until homogeneous. Store in glass bottle.

CAUTION — Extremely corrosive. Use effective fume removal device. Protect skin against exposure.

5.3.1.2 *Procedure* — Weigh sample containing 50 to 150 mg of sulphur into 250-ml beaker, and add 20 ml of 10 percent bromine in carbon tetrachloride. Mix by swirling beaker at 5 minutes interval during 30 minutes. Add 15 ml of concentrated nitric acid and mix as before. Evaporate to 1 to 2 ml on warm hot plate. Add 15 ml of concentrated hydrochloric acid and 10 ml of water. Evaporate just to dryness on warm hot plate or water bath. Add 10 ml of concentrated hydrochloric acid and 50 ml of water, heat to boiling, boil for 5 minutes, and filter through Whatman No. 42 filter paper or equivalent. Wash filter paper twenty times with 20 ml portions of hot water.

Alternatively, if the sample under test is rich in organic material, then boil gently for 30 to 45 minutes with 5 to 20 ml of nitric acid in a suitable flask (preferably Kjeldahl flask) to oxidize all easily oxidizable organic matter. Evaporate to 1 to 2 ml on warm hot plate. Repeat with another 10 ml of nitric acid if much organic matter remains. Evaporate just to dryness on warm hot plate. Cool and add 5 to 10 ml of 70 to 72 percent perchloric acid. Boil very gently until solution is colourless or nearly so and white dense fumes appear in the flask. Do not boil to dryness at any time. With samples containing large quantities of organic matter, temperature should be raised to fuming point (approximately 170°C) over a period of at least 1 hour. Cool slightly, add 50 ml of water and boil for a few minutes. Cool and filter through Whatman No. 42 filter paper or equivalent. Wash filter paper 3 times with 50 ml portions of the water.

5.3.1.3 Heat filtrate to boiling. Add 5 to 6 drops of barium chloride solution. After 1 minute add dropwise enough of barium chloride solution equivalent to expected sulphur content plus 5 ml excess. Digest on water bath for one hour. Remove it from hot plate and let the precipitate settle for 15 to 20 minutes. Filter immediately through Whatman No. 42 or prepared Gooch crucible, previously ignited and weighed. Wash with hot water until 10 ml washed water shows no precipitate with 3 ml of 1 percent silver nitrate solution. Dry and ignite at 800°C to constant mass. Cool in a desiccator and weigh.

5.3.1.4 *Calculations* — as in **5.3.2.2**.

5.3.2 *Free Sulphur*

5.3.2.1 *Procedure* — Extract 1 g sample with carbon disulphide in soxhlet apparatus, letting extraction thimble drain not less than 12 times. Transfer extract to 250 ml beaker using small lots for washing down. Evaporate carbon disulphide at room temperature under proper ventilation (*see* Note). Heat in oven for 20 minutes at 60 to 70°C, then cool to room temperature. Add 10 ml solution of bromine in carbon tetrachloride and allow it to stand for about 30 minutes stirring several times. Add 15 ml nitric acid, cover and let it stand for about 30 minutes, stirring several times. Evaporate on hot plate to about 5 ml. Add 20 ml of concentrated hydrochloric acid and evaporate to about 5 ml. Add about 50 ml of water, filter and wash with HCl (1 + 49). Add about 2 drops of bromophenol blue and then ammonium hydroxide to first colour change. Add concentrated hydrochloric acid dropwise until distinctly acidic, then 5 drops excess, dilute to 150 ml, heat to boiling point and add 10 percent barium chloride solution dropwise until about 50 percent excess is present. Cover beaker and digest on steam bath for more than 1 hour. Cool to room temperature and filter through asbestos on Gooch crucible previously ignited at 800°C or through Whatman No. 42 paper. Wash 10 times with hot water. Ignite in furnace at 800°C to constant weight. In case of filter paper, ignition is to be done on a previously ignited, weighed silica crucible.

NOTE — A caution from naked flame should be taken when carbon disulphide is evaporated.

5.3.2.2 *Calculation*

$$\text{Sulphur, percent by mass} = \frac{M_1 \times 0{\cdot}137\,4 \times 100}{M_2}$$

where

M_1 = mass in g of barium sulphate, and
M_2 = mass g of sample taken for the test.

5.3.3 *Turbidity Method*

5.3.3.1 *General* — The turbidity of a dilute barium sulphate suspension is difficult to reproduce. It is, therefore, essential to adhere rigidly to the experimental procedure detailed below. The velocity of the precipitation as well as the concentration of reactants, must be controlled by adding (after all the other components are present) pure solid barium chloride of definite grain size. The rate of solution of the barium chloride controls the velocity of the reaction. Sodium chloride and hydrochloric acid are added before the precipitation in order to inhibit the growth of microcrystals of barium sulphate, the optimum *p*H is maintained and minimises the effect of variable amounts of other electrolytes present in the sample upon the size of the suspended barium sulphate particles. A glycerol-alcohol solution helps to stabilise the turbidity. The reaction vessel is shaken gently in order to obtain a uniform particle size (each vessel should be shaken at the same rate and the same number of times). The unknown must be treated exactly like the standard solution. The intervals between the time of precipitation and measurement must be kept constant.

5.3.3.2 *Reagents*

a) *Standard sulphate solution* — Dissolve 1·814 1 of dry (Analytical Reagent Grade) potassium sulphate in distilled water and dilute to 1 litre in a volumetric flask. This solution contains 1·000 mg of sulphate ion per ml.

b) *Sodium chloride-hydrochloric acid reagent* — Dissolve 60 g of (Analytical Reagent Grade) sodium chloride in 200 ml of distilled water, add 5 ml of pure concentrated hydrochloric acid and dilute to 250 ml.

c) *Barium chloride* — Use crystals of (Analytical Reagent Grade) barium chloride that pass through a 20-mesh sieve and are retained by a 30-mesh sieve.

d) *Glycerol-alcohol solution* — Dissolve 1 volume of pure glycerol in 2 volumes of absolute ethanol.

5.3.3.3 *Procedure* — Run in 0·5, 1·0, 1·5, 2·0, 2·5 and 3·0 ml of standard potassium sulphate solution from a calibrated burette into separate 100 ml volumetric flasks. To each flask add 10 ml of the sodium chloride-hydrochloric acid reagent and 20 ml of the glycerol-alcohol solution, and dilute to 100 ml with distilled water. Add 0·3 g of the sieved barium chloride to each flask, stopper each flask and shake for 1 minute by inverting each flask once per second so that all the barium chloride should dissolve. Allow each flask to stand for 2-3 minutes and measure the turbidity in the EEL nephelometer (take care to avoid small air bubbles adhering to the walls of the matched test-tubes). Use the most concentrated solution as standard and, by means of the sensitivity control, adjust the galvanometer reading to 100 divisions. Prepare a 'blank solution', repeat the above sequence of operations but do not add any sulphate solution. Place the blank solution in the nephelometer and adjust to zero reading of the galvanometer suspension. Check the reading of the most turbid solution and adjust any deviation from 100 by means of the sensitivity control. Repeat the measurement with the five other standard sulphate solution. Plot the galvanometer reading against the sulphate-ion content per ml.

5.3.3.4 Add to a suitable aliquot of the acid solution prepared in **4.1** or **4.2** necessary amount of hydrochloric acid so that each millilitre contains not more than 50 micrograms of sulphur. Carry out the whole procedure as described in **5.3.3.3**. Measure the turbidity and use calibration curve to obtain the net measure of sulphur in the sample.

6. METHODS OF TEST FOR MICRONUTRIENTS

6.1 Determination of Boron

6.1.0 *General* — Three methods are prescribed. The method shall be selected depending upon the quantity of boron to be estimated. All glasswares used in boron estimation must be of 'soda glass' or specially made 'boron free' glass. Good quality heat resistant polyethylene wares or silica vessels are also quite suitable.

6.1.1 *Method A (Carmine Method)*

6.1.1.1 *Range* — Boron content of up to 25 ml microgram in the 2 ml taken for colour development.

6.1.1.2 *Outline of the method* — After destruction of organic matter by ashing in the presence of calcium hydroxide, boron is determined absorptiometrically as its coloured complex with caramine.

6.1.1.3 *Reagents* — In order to avoid contamination of reagents, they shall not be kept in bottles made of borosilicate glass. The reagents used shall also be boron-free. If it is found that the reagents blank gives a high value compared to the test result, the test shall be repeated using a fresh supply of boron-free reagents.

a) *Calcium hydroxide solution* — saturated. Use pure calcium hydroxide for preparation.

b) *Dilute hydrochloric acid* — 2 N (*see* IS : 268-1976*).

c) *Concentrated sulphuric acid* — (*see* IS : 266-1977†).

d) *Carmine solution* — Mix 0·1 g of carmine with 100 ml of concentrated sulphuric acid and leave the mixture overnight, or until the dye is completely dissolved.

e) *Standard boron solution* — Dissolve 0·571 6 g of recrystallized boric acid in water, and dilute the solution to 1 litre. One millilitre of the solution is equivalent to 100 micrograms of boron.

6.1.1.4 *Procedure* — Weigh accurately a suitable quantity 1 to 2 g of the sample into a 25 ml silica dish, add 20 ml of saturated calcium hydroxide solution (and, if necessary, sufficient solid calcium hydroxide to react with any ammonium salts present), evaporate the mixture to dryness, and ash the residue as completely as possible in a muffle furnace at 450 to 500°C for three to four hours. Cool and stir thoroughly with 10 to 15 ml of dilute hydrochloric acid. Filter into a 25 ml volumetric flask, wash the residue with water, and dilute the filtrate to 25 ml with water. Alternatively, spin the mixture in a centrifuge for 5 to 10 minutes, and decant the supernatant liquid into a 25-ml calibrated flask. Wash the solid in the centrifuge with two successive 5 ml portions of water, stirring up the residue well after each addition of water. Spin the mixture after each addition, and decant the washings into the flask Finally dilute to the mark with water.

Transfer 2 ml of the solution prepared as in **6.1.1.4** to a beaker made of soda glass polyethylene cautiously, add 10 ml of concentrated sulphuric acid, mix, and cool. Add 10 ml of carmine solution, mix, and set aside for 45 minutes for colour development.

Carry out a blank determination with all the reagents used.

Measure the optical densities of the test and blank solutions in spectrophotometer at a wavelength of 585 nm, using 1 cm quartz cells, with water, in the comparison cell. Read the number of micrograms of boron

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*Specification for hydrochloric acid (*second revision*).

†Specification for sulphuric acid (*second revision*).

solutions from a previously prepared calibration graph and so obtain the net content of boron in the sample. The result may be expressed as micrograms of boron per gram of sample.

For preparing the calibration graph, measure appropriate amounts of standard boron solution, covering the range 0 to 312 micrograms of boron, by diluting 0, 2·5, 5·0, 7·5, 10·0 and 12·5 ml of standard boron solution to 20 ml with water in a series of calibrated flask By pipette measure a 5 ml quantity of saturated calcium hydroxide solution to each dish, and carry out the whole procedure, commencing at the evaporation to dryness, as described in **6.1.1.4.** Measure the optical densities of the solutions, and plot a graph relating to the optical densities to the number of micrograms of boron.

6.1.2 *Method B* (*Curcumin Oxalic Acid Reagent Method*)

6.1.2.1 *Outline of the method* — After destruction of organic matter, sample solution is treated with calcium hydroxide and finally boron is determined absorptiometrically as its coloured complex with curcumin-oxalic acid.

6.1.2.2 *Reagents*

a) *Calcium hydroxide suspension* — Dissolve 0·4 g of calcium hydroxide in 100 ml of distilled water.

b) *Dilute hydrochloric acid* — 2·5 N (*see* IS : 268-1976*).

c) *Standard boron solution*

Solution A — Dissolve 0·572 g of AR boric acid in distilled water and dilute the solution to one litre in a volumetric flask, 1 millilitre of this solution contains 0·1 mg of boron (100 micrograms of B/ml).

Solution B — Transfer 10 ml of solution A to a one litre volumetric flask and dilute to mark. 1 millilitre of this solution contains 0·001 mg of boron (1 micrograms B/ml).

d) *Bromothymol blue indicator*

e) *Phenolphthalein indicator*

f) *Curcumin-Oxalic acid reagent* — Dissolve 0·04 g of finely ground curcumin and 5 g of AR oxalic acid in 100 ml of 95 percent ethyl alcohol. Store this solution in refrigerator.

6.1.2.3 *Procedure* — Weigh accurately 1 to 2 g of the sample into a 25 ml silica dish. Add 5 ml of calcium hydroxide suspension and evaporate

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*Specification for hydrochloric acid (*second revision*).

the contents to dryness on water bath. Allow the dish to cool and then add 1 drop of phenolphthalein indicator. Add 2·5 N HCl dropwise until the colour of indicator disappears. Add 0·5 ml of acid in excess. Add 4 ml of curcumin-oxalic acid reagent and rotate the dish to bring the solution into contact with all parts of the sample. Place the dish on a mesh support about 1 cm below the surface of water in a water bath maintained at 55 ± 3°C. Allow the contents to dry and keep for another 15 minutes on water bath.

Moisten the residue with 95 percent ethyl alcohol. After few minutes transfer the contents to a 15 ml centrifuge tube with additional amount of ethyl alcohol using a policeman to aid in the transfer. Centrifuge for 10 minutes at 1 500 rpm. Decant the supernatant solution into a 25 ml volumetric flask, add 10 ml of ethyl alcohol to the tube, shake well and centrifuge once again. Decant into the same volumetric flask and make up the volume to 25 ml, carry out a blank determination with all the reagents used.

Measure the absorbance of the test solution at 540 nm. Read the number of micrograms of boron solutions from a previously prepared calibration graph and so obtain the net content of boron in the sample. The result may be expressed as micrograms of boron per gram of the sample.

For preparing calibration graph, pipette out 0, 1, 2, 3, 4 and 5 ml aliquots of standard solution B into separate evaporating dishes (75 mm size) and carry out the whole procedure, as in **6.1.2.3** commencing at 'Add 5 ml of calcium hydroxide........'. Measure the optical densities of the solutions and plot a graph relating to the optical densities to the number of micrograms of boron.

6.1.3 *Method C (Electrometric Titration Method)*

6.1.3.1 *Range* — Boron content between 1 and 10 mg in the final aliquot (corresponding to fertilizers containing 0·1 to 1·0 percent of boron).

6.1.3.2 *Outline of the method* — In this method, after removal of phosphate by the addition of lead nitrate and removal of the excess lead with sodium bicarbonate, the borate in the filtrate is titrated by an 'identical *p*H' technique in the presence of mannitol.

6.1.3.3 *Apparatus* — A suitable *p*H meter, with glass electrode.

6.1.3.4 *Reagent* — In order to avoid contamination of reagents, they shall not be kept in bottles made of borosilicate glass. The reagents used shall also be boron free. If it is found that the blank gives a high value compared to the test result, the test shall be repeated using a fresh supply of boron-free reagents.

a) *Concentrated nitric acid* — (*see* IS : 264-1976*).

b) *Lead nitrate solution* — Dissolve 10 g of lead nitrate in 5 ml of concentrated nitric acid, with slight warming, if necessary, and dilute to 100 ml with water.

c) *Sodium bicarbonate* (*solid*)

d) *Methyl red indicator solution* — Dissolve 0·1 g of methyl red in 50 ml of ethanol and dilute to 100 ml with water.

e) *Dilute hydrochloric acid* — approximately 4 N, 0·5 N and 0·02 N.

f) *Sodium hydroxide solution* — approximately 0·5 N, made from carbonate-free sodium hydroxide and carbon dioxide-free water. Store the solution in a bottle fitted with a guard tube containing soda lime.

g) *Standard sodium hydroxide solution* — 0·02 N, carbonate-free, made by diluting sodium hydroxide solution with carbon dioxide free water. Standardize against boric acid solution following the procedure given in **6.1.3.5**. Store the solution in a bottle fitted with a guard tube cantaining soda lime.

h) *Mannitol* — reagent grade.

6.1.3.5 *Procedure* — Transfer 2·5 g of sample to a 500-ml beaker, add 50 ml of water and 3 ml of concentrated hydrochloric acid and stir. Warm the solution to between 80 and 90°C (do not boil, as boric acid is volatile in steam), and slowly add from a burette, with continuous stirring adn maintaining liquid hot during the addition, 10 ml of lead nitrate solution or 1 ml of it for each 1·2 percent of P_2O_5 if the P_2O_5 content of the sample is known and is more than 12 percent. Add sodium bicarbonate, a little at a time, until the solution approaches neutrality (often observed by formation of a white precipitate in addition to the insoluble matter already present). Add a few drops of methyl red indicator and continue adding sodium bicarbonate gradually until the solution is just alkaline to methyl red. Keep the mixture hot, but not boiling, for 30 minutes, adding additional quantities of sodium bicarbonate if needed, to keep the same indicator colour. If the indicator is bleached by nitrates present in the sample, add more of it; if the colour is obscured by organic matter, use external spot tests to forjudging neutralization. After neutralization and heating 40 to 50 ml of the solution should remain. Filter the hot solution into a 250-ml beaker and wash the residue thoroughly with hot water. Acidify the filtrate with a few drops of dilute hydrochloric acid and boil briefly to expel most of the carbon dioxide. Neutralize the hot solution with 0·5 N sodium hydroxide solution, and re-acidify with 0·5 N hydrochloric

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*Specification for nitric acid (*second revision*).

acid, using 0·3 to 0·5 ml in excess. Dilute to about 150 ml and boil gently for a few minutes to expel remaining carbon dioxide. Cool to room temperature in running water.

Roughly neutralize the solution using 0·5 N sodium hydroxide solution, and place in the solution the electrodes of a suitable *p*H meter, and adjust the *p*H to exactly 6·3 by adding 0·02 N sodium hydroxide solution or 0·02 N hydrochloric acid as required. Add 20 g of mannitol, and again bring *p*H to 6·3 with standard sodium hydroxide solution. Continue to add 10 g portions of mannitol and to readjust the *p*H to 6·3 until, after the final addition of mannitol, the *p*H remains constant at 6·3 (for samples containing up to 0·5 percent of boron, 20 g of mannitol is usually sufficient). The total amount of standard sodium hydroxide solution used after the first addition of mannitol corresponds to the amount of boron present in the solution.

Carry out a blank determination using a chemically similar type of fertilizer to which boron has not been added.

6.1.3.6 *Calculation*

Boron content, percent by mass = $0{\cdot}216\ (V_1 - V_2)$

where

V_1 = volume in ml of standard sodium hydroxide solution used with the sample; and

V_2 = volume in ml of standard sodium hydroxide solution used in the blank determination.

6.2 Determination of Copper

6.2.1 *Range* — For copper contents up to 50 micrograms in the final aliquot.

6.2.2 *Outline of the Method* — In this method, after separation from other compounds, copper is extracted at *p*H 8·5 as its diethyldithiocarbamate complex in carbon tetrachloride; interference from other metals, except bismuth and tellurium, is prevented by the addition of EDTA and citrate. The golden brown copper complex is then determined absorptiometrically. Bismuth interferes, but additional procedural details are given if this element is found to be present.

6.2.3 *Reagents*

6.2.3.1 *Distilled water* — Water distilled from all-glass apparatus (preferably double distilled) shall be used for preparing the reagents and the procedure.

6.2.3.2 *Carbon tetrachloride* — re-distilled.

6.2.3.3 *Sodium diethyldithiocarbamate solution*— Dissolve 1 g of sodium; diethyldithiocarbamate ($C_5H_{10}NaNS_2 \cdot 3H_2O$) in 100 ml of distilled water filter the solution if it is not clear. Store in dark in a refrigerator, and discard after 7 days.

6.2.3.4 *EDTA-citrate solution* — Dissolve 20 g of ammoniumcitr ate and 5 g of the disodium salt of ethylenediamine tetra-acetic acid (EDTA) in distilled water, and dilute to 100 ml with distilled water. To purify, add 0·1 ml of sodium diethyl dithiocarbamate solution, and extract with carbon tetrachloride. Add a further quantity of carbamate to ensure that it is in excess.

6.2.3.5 *Thymol blue indicator solution* — Triturate to dissolve 0·1 g of thymol blue in 2·5 ml of 0·1 N sodium hydroxide solution, and dilute to 100 ml with distilled water.

6.2.3.6 *Ammonium hydroxide* — 6 N approximately. This may be prepared by passing gaseous ammonia through distilled water, or by purifying ammonia solution as described for EDTA-citrate solution.

6.2.3.7 *Sulphuric acid* — 2 N (*see* IS : 266-1977*).

6.2.3.8 *Standard copper solution* — (1 000 µg copper per ml). Dissolve 1 g of pure copper metal in minimum amount of nitric acid and add 5 ml of hydrochloric acid. Evaporate almost to dryness and dilute to 1 litre with hydrochloric acid (0·1 N).

6.2.3.9 *Potassium cyanide solution* — 5 percent. This extract reagent is required only if bismuth is present.

> CAUTION — Potassium cyanide is extremely poisonous and should be used very carefully. Wear resistant rubber gloves when handling.

6.2.4 *Procedure*

6.2.4.1 Transfer a suitable aliquot of the acid solution prepared as in **4.1** or **4.2** containing not more than 50 micrograms of copper to a separating funnel, and add 10 ml of EDTA-citrate solution, 2 drops of thymol blue indicator solution, and sufficient ammonium hydroxide until the solution is coloured green or bluish/green. Cool, add 1 ml of sodium carbamate solution from a burette and 15 ml of carbon tetrachloride; stopper the funnel, shake vigorously for 2 minutes, and allow the layers to separate. Place a piece of cotton wool in the stem of the separating funnel, and run off the carbon tetrachloride layer into a dry spectrophotometer cell, taking care to avoid undue exposure of the solution to light.

6.2.4.2 Carry out a blank determination on all reagents used.

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*Specification for sulphuric acid (*second revision*).

6.2.4.3 Immediately, measure the optical densities of the test and blank solutions in a spectrophotometer at a wavelength of 436 nm, or in an absorptiometer using a suitable blue filter, use carbon tetrachloride in the comparison cell. Read the number of micrograms of copper equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of copper in the sample. Establish the calibration graph as follows :

> To a series of separating funnels transfer 10 ml of EDTA citrate solution and the following amounts of standard copper solution and of sulphuric acid :
>
> | | | | | | | | | | |
> |---|---|---|---|---|---|---|---|---|---|
> | Copper solution | 0 | 1 | 2·5 | 5 | 10 | 15 | 20 | 25 | ml |
> | Sulphuric acid | 25 | 24 | 22·5 | 20 | 15 | 10 | 5 | 0 | ml |
>
> Proceed as in **6.2.4.1** commencing at the addition of 2 drops of thymol blue indicator. Measure the optical densities of the solutions, and construct a graph plotting the optical densities against micrograms of copper.

6.2.4.4 *Interference of bismuth* — Bismuth is also extracted by the carbamate reagent under the conditions described, if bismuth is present, the above determination (**6.2.4.2**) should be repeated, 10 ml of potassium cyanide solution being added (from burette) before the sodium carbamate solution, and the second optical density reading should be subtracted from the first to get the optical density.

6.3 Determination of Iron

6.3.0 *General* — Two methods are given below. The method may be selected depending upon the quantity of iron estimated.

6.3.1 *Method 1*

6.3.1.0 *Range* — For iron contents up to 1 mg in the final aliquot.

6.3.1.1 *Spectrophotometric procedure* — In test solution prepared as given under **4.1** or **4.2**, iron is determined absorptiometrically as its red complex with *o*-phenanthroline.

6.3.1.2 *Reagents*

a) *Bromophenol blue indicator solution* — 0·4 percent solution in 95 percent ethanol.

b) *Sodium citrate solution* — 25 percent.

c) *Hydroquinone solution* — 1 percent in an acetic acid buffer of *p*H 4·5 obtained by mixing 65 ml of 9·1 M acetic acid with 35 ml of 0·1 M sodium acetate solution.

d) *o-Phenanthroline solution* — 0·25 percent solution in 25 percent ethanol.

e) *Standard iron solution* — Dissolve 0·700 2 g of ferrous ammonium sulphate (analytical grade reagent) in water containing 2 ml of perchloric acid, and dilute to 100 ml with water. Dilute 10·0 ml of this solution to 100 ml at 20°C with water immediately before use. One millilitre of the solution contains 100 micrograms of iron.

f) *Dilute hydrochloric acid* – 1 : 3 (*v/v*).

6.3.1.3 *Procedure* – To 5 ml of the acid solution prepared in **4.1** or **4.2** add bromophenol blue indicator solution, and titrate with sodium citrate solution until the yellow colour changes to blue.

To another 5 ml of the acid solution in a 25-ml calibrated flask, add 1 ml of hydroquinone solution, 3 ml of *o*-phenanthroline solution and an amount of sodium citrate solution equivalent to the above titration, and then dilute with water to 25·0 ml. Allow the solution to stand for 1 hour.

Carry out a blank determination on all the reagents used.

Measure the optical densities of the test and blank solutions in a spectrophotometer at a wavelength of 510 nm, in an absorptiometer using a suitable green filter, use 4 cm or 1 cm cells according to the depth of colour, with water in the comparison cell. Read the number of micrograms of iron equivalent to the observed optical densities of the test and blank solutions from a previously preparedc alibration graph, and so obtain the net measure of iron in the sample.

Establish the calibration graph as follows :

Measure appropriate amounts of standard iron solution into a series of 100-ml calibrated flasks. To each add 40 ml of dilute hydrochloric acid, and dilute to 100 ml with water. Using 5 ml aliquots, proceed as for the test solution. Measure the optical densities of the solutions, and construct a graph relating the optical densities to the number of micrograms of iron.

6.3.2 *Method 2*

6.3.2.1 *Reagents*

a) *Diphenylamine solution* – Dissolve 1 g in 100 ml of concentrated sulphuric acid.

CAUTION – Diphenylamine may be harmful. Use gloves and goggles to avoid contact with skin and eyes.

b) *Diphenylamine sulphonate solution* — Dissolve 0·5 g in water in 100-ml volumetric flask and dilute it to volume.

c) *Potassium dichromate standard solution* — 0·1 N and 0·01 N.

d) *Mercuric chloride saturated solution* — Shake excess of mercuric chloride (H_gCl_2) with water and let it settle.

> CAUTION — Mercuric salts are quite toxic and mostly water soluble. Useskin and respiratory protection when handling these salts and their concentrated solutions.

e) *Stannous chloride solution* — Dissolve 20 g stannous chloride ($SnCl_2 \cdot 2H_2O$) in 20 ml of hydrochloric acid, warm gently. Add 20 ml of water and dilute to 100-ml with hydrochloric acid (1 + 1). Keep warm until clean then add few granules of tin and the solution should be prepared at intervals. Dispense from droping bottle.

6.3.2.2 *Preparation of sample solution*

a) *Suitable for all fertilizers* — Take 1 g sample and boil gently 20 to 45 minutes with 20 to 30 ml of concentrated nitric acid in suitable flask (preferably Kjeldahl flask for samples containing large amount of organic matter) to oxidize all easily oxidizable matter. Cool and add 5 to 10 ml of perchloric acid. Boil very gently until solution is colourless or nearly so and dense white fumes appear in flask. *Do not boil to dryness at any time.* Hold it for 1 hour at about 170°C to remove nitric acid completely. Cool slightly, add 50 ml of water, and boil for few minutes. Dilute it to 250 ml.

b) *Suitable for soluble salts and oxides* — Dissolve 1 g sample in 10 ml of concentrated hydrochloric acid. Warm gently and dilute it to 250 ml.

6.3.2.3 *Procedure* — Heat aliquot as sample solution (100 ml and 50 ml, respectively for sample containing less than 0·5 and 0·5 to 4·0 percent iron) to boiling point. Add few drops diphenylamine sulphonate solution, then stannous chloride solution dropwise until violet colour is discharged and 2 drops excess (usually 1 to 6 drops are required. Larger amount may be used with samples containing large amount of iron). If reduction does not occur, discard and proceed as follows with second aliquot.

Add few granules of zinc, boil for few minutes, and either filter off excess zinc, washing with hot water, or let zinc dissolve. Heat to boiling point and furnish reduction with stannous chloride and diphenylamine sulphonate indicator as above. Add 10 ml of hydrochloric acid (1 + 1). Adjust volume to 75 to 110 ml with water. Cool rapidly in cool water. Add 10 ml of standard mercuric chloride solution, swirl gently, add 5 ml of phosphoric acid, and titrate immediately (small amount of mercurous chloride must precipitate to ensure complete reduction).

Add 2 drops diphenylamine indicator by pipette (no more : excess will interfere with end point if amount of iron is small). Titrate with 0·01 N potassium dichromate solution. Since end point may be difficult to see with very small amount of iron, approach end point slowly, allowing few seconds for colour to develop. Titrate to permanent blue (sometimes green with very small amount of iron). For sample containing more than 4 percent iron, use 0·1 N potassium dichromate for titration. One ml of 0·1 N potassium dichromate is equal to 0·005 58 g of iron and 1 ml of 0·01 N of potassium dichromate is equal to 0·000 558 g of iron.

6.4 Determination of Manganese

6.4.0 *General* — Two methods are given below. The method shall be selected depending upon the quantity of manganese estimated.

6.4.1 *Method 1*

6.4.1.0 *Range* — For manganese contents up to 70 micrograms in the final aliquot.

6.4.1.1 *Outline of the method* — After destruction of the organic matter, manganese is oxidized to permanganate by means of periodate in acid solution, and the permanganate is then determined absorptiometrically.

6.4.1.2 *Reagents*

a) *Ortho-phosphoric acid*

b) *Potassim periodate*

c) *Sodium oxalate* — 0·1 N.

d) *Standard manganese solution* — Dissolve 0·288 g of potassium permanganate (analytical reagent grade) in 100 to 200 ml of water, add 5 ml of dilute sulphuric acid 1 : 3 (*v/v*), and dilute to 1 litre at 20°C with water, standardize by titration with standard sodium oxalate and adjust so that 1 ml is equal to 0·1 mg of manganese. Draw out 25 ml of this solution warm and add a pinch of sodium bisulphite powdered first to discharge the pink colour. Cool and dilute to 250 ml. One millilitre of the final solution contains 10 micrograms of manganese.

6.4.1.3 *Procedure* — Transfer a suitable aliquot of the acid solution prepared in **4.1** or **4.2** containing not more than 70 micrograms of manganese, to a small beaker, evaporate to dryness at a low heat on a hot-plate, cool, add 3 ml of ortho-phosphoric acid, and warm to dissolve the residue. Cool, add carefully 4 ml of water warm again, and transfer the solution to a 10 ml calibrated stoppered tube. Wash the beaker with further 3 ml of warm water, adding this to the content of tube. Add 0·5 g of potassium periodate boil for 1 minute, cool, adjust the volume of the solution to just above the 10 ml mark with water, heat the loosely stoppered tube in a boiling water-bath for 30 minutes, cool, and adjust the volume to the mark with water.

Carry out a blank determination on all the reagents used.

Measure the optical densities of the test and blank solution in a spectrophotometer at a wavelength of 526 nm using 1 cm cells, with water in the comparison cell. Read the number of micrograms of manganese equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of manganese in the sample.

For establishing the calibration graph, measure appropriate amounts of dilute standard manganese solution, covering the range 0 to 70 micrograms of manganese into a series of 10 ml stoppered tubes. To each, add 3 ml of ortho-phosphoric acid and proceed as in **6.4.1**, commencing at 'Add 0·05 g of potassium periodate............'. Measure the optical densities of the solutions, and construct a graph relating the optical densities to the number of micrograms of manganese.

6.4.2 *Method 2 (Bismuthate method)*

6.4.2.1 *Reagents*

a) *Sodium bismuthate powder* — 80 percent sodium bismuthate (NaB_2O_3) containing less or equal to 0·000 5 percent manganese and less or equal to 0·002 percent chloride.

b) *Potassium permanganate standard solution* — 0·091 N. Dissolve 2·876 g of potassium permanganate in water and dilute to 1 litre. One ml of this solution is equal to 1 mg of manganese. Standardize it against sodium oxalate solution.

c) *Ferrous sulphate standards solution* — 0·091 0 N. Dissolve 25·3 g ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), 25 ml of sulphuric acid, and 25 ml of phosphoric acid in water and dilute it to 1 litre. One ml of this solution is equal to 1 mg of manganese. Standardize with potassium permanganate at the time of actual use. Place measured portion about equivalent to maximum quantity of manganese to be determined in an Erlenmeyer flask containing 200 ml of cold sulphuric acid (3 + 97), and titrate with potassium permanganate standard solution to the first appearance of pink colour for half a minute.

6.4.2.2 *Procedure* — To 1 g sample in Erlenmeyer flask (preferably 300 ml), add 5 to 10 ml of nitric acid and 7 ml of concentrated sulphuric acid. Evaporate on hot plate to white fumes, and repeat until organic matter is destroyed. Cool and add 200 ml of water, 10 ml concentrated nitric acid, and just enough sodium bismuthate to give strong permanganate colour, or if quantity of manganese is small, add slight excess of sodium bismuthate. Boil gently 2 to 3 minutes. If permanganate colour disappears, cool somewhat, and repeat bismuthate treatment. (Permanent permanganate colour indicates sufficient excess of bismuthate.) Add saturated sodium bisulphite solution dropwise while stirring until manganese compounds are reduced and solution clears. Avoid large excess. Boil gently for 2 to 3 minutes. Cool to room temperature, and dilute to about 100 ml. If solution contains less than 40 mg of manganese, proceed as above, if more than 40 mg manganese, transfer to 200-ml volumetric flask, add 5 ml of concentrated sulphuric acid and 10 ml of concentrated nitric acid, cool, dilute it to volume and mix. Pipette aliquot containing less or equal to 40 mg manganese into Erlenmeyer flask and dilute to 100 ml with solution containing 5 ml of concentrated sulphuric acid and 10 ml of concentrated nitric acid in 100 ml.

Before continuing preparation suction filters of asbestos, wash with sulphuric acid (3 + 97) and then with water, (Glass filter tubes with perforated porcelain discs to support asbestos and connected with suction flask are satisfactory. Manganese solution must not contact rubber). Then complete determination without interruption. To manganese solution at 20 to 30°C add at least 0·025 g of sodium bismuthate for each 10 mg of manganese. Swirl contents of flask for 1 minute, add 10 ml of water, and mix. Filter with suction through filter paper and wash with cold sulphuric acid (3 + 97) until washings show no pink tint. Disconnect suction flask, and add ferrous sulphate at least 10 percent excess with 1 ml of minimum excess. Titrate excess ferrous sulphate with potassium permanganate standard solution to faint pink. From potassium permanganate equivalence of ml of ferrous sulphate solution used, substract the potassium permanganate used in blank titration.

6.5 Determination of Molybdenum

6.5.1 *Range* — For molybdenum contents up to 30 micrograms in the final aliquot.

6.5.2 *Outline of the Method* — Follow the standard procedure to destroy organic matter as in **4.1** or **4.2**, molybdenum in the presence of a reducing agent gives an orange-coloured complex with potassium thiocyanate which is soluble in organic solvent such as *iso*-amyl alcohol. It is extracted from aqueous solution and determined absorptiometrically.

6.5.3 *Reagents*

6.5.3.1 *Dilute hydrochloric acid* — 1·0 N (*see* IS : 265-1976*).

6.5.3.2 *Potassium thiocyanate solution* — 40 percent.

6.5.3.3 *Stannous chloride solution* — Suspend 40 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in 20 ml of 6·5 N hydrochloric acid, add water to dissolve warm if necessary and dilute the solution to 100 ml with distilled water; filter, if turbid.

6.5.3.4 *Solvent mixture* — Mix equal volumes of carbon tetrachloride and *iso*-amyl alcohol (both analytical reagent grade).

6.5.3.5 *Standard molybdenum solution* — Dissolve 0·184 g of ammonium molybdate [$(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$] in water, and dilute the solution to 1 litre at 20°C with water. Dilute. 10 ml of this solution to 1 litre at 20°C with water. One millilitre of the solution contains 1 microgram of molybdenum.

6.5.4 *Procedure*

6.5.4.1 Transfer a suitable aliquot (usually 5 to 10 ml) of the acid solution prepared in **4.1** or **4.2** to a 125-ml separating funnel, add sufficient dilute hydrochloric acid to bring the volume to 50 ml (*see* Note 1 and 2), then add 2 ml of potassium thiocyanate solution, and mix. Add 1 ml of stannous chloride solution, and mix again to develop the orange coloured complex. Add exactly 5 ml of solvent mixture, shake vigorously for 2 minutes, allow to separate for 15 minutes, and filter the lower layer through a 7 cm filter paper into a small stoppered funnel directly into the spectrophotometer cell; a few milligrams of solid stannous chloride may be placed in the folded filter-paper before filtering to ensure that a large excess of reducing agents present in order to discharge completely the red colour due to iron thiocyanate.

NOTE 1 — In order that the colour development may be complete, 5 mg of iron should be present in solution and, if this condition is not inherent in the material under examination, then a suitable quantity of iron be added.

NOTE 2 — The acidity of the final solution should not exceed 1·5 N with respect to hydrochloric acid; with more strongly acid conditions, fading of the colour will occur.

6.5.4.2 Carry out a blank determination on all reagents used.

6.5.4.3 Measure the optical densities of the test and blank solutions in a spectrophotometer at a wavelength of 470 nm, using 1 cm cells, with water in the comparison cell. Read the number of micrograms of molybdenum equivalent to the observed densities of the test and blank solutions previously prepared calibration graph, and so obtain the net measure of molybdenum in the sample.

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*Specification for hydrochloric acid (*second revision*).

6.5.4.4 For preparing the calibration graph, transfer suitable amounts of the standard molybdenum solution, covering the range 0 to 30 micrograms of molybdenum, to a series of 124-ml separating funnels, and proceed as for the test solution. Measure the optical densities of the solutions, and construct a graph relating optical densities to the number of micrograms of molybdenum.

6.6 Determination of Zinc

6.6.0 *General* — Two methods for determining zinc content are given in this section. The method given in **6.6.1** is the referee method and shall be used in case of any dispute. The method given in **6.6.2** shall be an alternative ones which shall be used for routine analysis.

6.6.1 *Atomic Absorption Spectrophotometric Method*

6.6.1.1 *Reagents*

a) *Dilute nitric acid* — (1 : 1).

b) *Dilute sulphuric acid* — 10 percent.

c) *Standard zinc solution* — Weigh 1·000 0 g of pure zinc on a clean watch glass, dissolve in 10 ml of hydrochloric (1 : 1) and wash it down to one-litre flask through the funnel with distilled water. Add two ml of dilute nitric acid and make the volume up to the mark. Stopper the flask and shake well. This is 1 000 mg/litre zinc solution A. This solution should be stored in a clean resistant glass bottle. Dilute 10 ml of 1 000 mg/litre solution of zinc standard solution A to 1 000 ml/litre standard zinc solution, designated as solution B.

d) *Glass distilled water* — *p*H 2·5 ± 0·5. Add one ml of 10 percent sulphuric acid to one litre of glass distilled water and adjust the *p*H to 2·5 with a *p*H meter using sulphuric acid or sodium hydroxide. For regular use 5 to 10 litres of the acidified water can be prepared and stored in hard glass or polyethylene bottles.

e) *Preparation of working standards* — Pipette the following amounts of standard solution B in 50-ml volumetric flasks and make up to the mark with acidified water. Prepare the standard in duplicate.

Flask No.	*Volume of Standard Solution B* ml	*Concentration of Zinc after Making Volume to 50 ml* (mg/litre)
(1)	(2)	(3)
1	0·0	0·0
3	2·0	0·4
5	4·0	0·8
7	7·0	1·4
9	10·0	2·0

Stopper the flasks and shake well. Fresh set of standard should be prepared every time with a new lot of acidified water.

6.6.1.2 *Procedure*

a) *Preparation of zinc sulphate fertilizer samples* — Transfer 0·25 g of the material weighed on a small watch glass to one-litre volumetric flask through the funnel with glass distilled water and dissolve the material by shaking. Add a few drops of dilute hydrochloric acid if turbidity is noticed. Make the volume to mark with glass distilled water.

b) Dilute 5 ml of the above solution taken in 250-ml volumetric flask and make the volume with the acidified water. After thorough shaking filter through Whatman No. 42 dry filter paper in dry clean flasks. The flasks should be rinsed with small portion of the filtrate, to be discarded and then filtration should be continued.

c) *Aspirating or atomizing* — Aspirate the standard and the filtered samples solution on atomic absorption spectrophotometer at a wavelength of 213·8 nm (zincline of the equipment).

6.6.1.3 *Procedure* — Prepare a standard curve of known concentrations by plotting the absorbance values of zinc on Y-axis against their respective concentration on X-axis. Calculate the percentage zinc in fertilizer by multiplying zinc concentration value obtained from standard curve by 20.

Mass of the fertilizer sample taken = 0·25 g
Prepared sample solution = 1 000 ml
Further dilution made = 50 times
Reading of the samples from ASS = Y
Corresponding concentration value of zinc from standard curve against Y absorbance = X mg/litre
Percentage of zinc (as Zn) in the sample = 20 (X)

6.6.2 *Modified (Ethylene Diamine Tetraacetate) EDTA Titration Method*

6.6.2.1 *Outline of the method* — Zinc is titrated against EDTA solution. The initial separation of zinc from impurities by zinc sulphide precipitation is retained. Interference may be expected from other metallic cations which are likely to be precipitated with H_2S.

6.6.2.2 *Reagents*

a) *Dilute sulphuric acid* — (1 : 100).

b) *Ammonium sulphate* — AR grade.

c) *Diammonium hydrogen phosphate solution* — 10 percent (*m/m*).

d) *Hydrochloric acid* — (1 : 1).

e) *Sodium hydroxide solution* — 1 N (standardized again 0·2 N H_2SO_4).

f) *Methyl orange indicator* — 0·05 percent.

g) *Methyl red indicator* — Dissolve 0·1 g of methyl red in 50 ml of ethanol and dilute to 100 ml with water.

h) *Hydrogen sulphide generator* — Kipp's apparatus using ferrous sulphide.

j) *Ethylene diamine tetraacetate (EDTA) solution* — 0·1 **M.** Dissolve 37·23 g of disodium dihydrogen ethylene diamine tetraacetate dihydrate (AR grade) in water and dilute to 1 litre in a volumetric flask with redistilled or deionised water.

k) *Ethylenediamine tetraacetate (EDTA) solution* — 0·01 **M.** Pipette out 25 ml of standardised EDTA solution (0·1 N) and make it up to 250 ml.

m) *Zinc ion solution* — 0·1 **M.** Dissolve completely 1·634 5 g accurately weighed zinc metal granulated (99·9) percent purity in dilute hydrochloric acid, nearly neutralise the resulting solution with sodium hydroxide solution and dilute to 250 ml in a volumetric flask with distilled water.

n) *Buffer solution* — *p*H 10. Add 142 ml of concentrated ammonia solution (relative density 0·88 to 0·90) to 17·5 g of ammonium chloride AR grade and dilute to 250 ml with distilled water.

p) *Eriochrome black T indicator* — Dissolve 0·2 g of the solid in 15 ml of triethanolamine and 5 ml of absolute ethanol.

6.6.2.3 *Procedure*

a) *Standardization of EDTA solution* — Dilute 25·0 ml of zinc ion solution to 100 ml with distilled water, add 2 ml of the buffer solution and a few drops of the eriochrome black T indicator. Titrate with the EDTA solution until the colour changes from wine red to blue. Calculate 1 ml EDTA equivalent to zinc.

b) Weigh accurately 7·5 g of the sample and dissolve it in water. Add 2 ml of dilute hydrochloric acid and make up to 250 ml. Filter through a dry filter paper (Whatman No. 42) in a dry beaker rejecting first 100 ml of the filtrate. Pipette out 25 ml of made up solution in a conical flask. Acidify with dilute sulphuric acid adding 1 to 2 drops of methyl orange indicator. The *p*H of solution has to be carefully adjusted between 2 to 3. (Solubility product of zinc sulphide is reported in literature as 1×10^{-24} to

8×10^{-26}) hence it is highly insoluble and can be precipitated in acid media where other metals of same group like cobalt, nickel and manganese do not precipitate. To obtain complete precipitation, add 6 to 8 g of ammonium sulphate which acts as coagulation agent. Fit the conical flask with a two hole rubber stopper carrying inlet tube extending to the bottom of the flask and an outlet tube extending up to the bottom of the stopper. Pass a steady stream of hydrogen sulphide through the solution rapidly for half an hour at room temperature. Allow the precipitate to settle for 15 minutes. Then filter through Whatman No. 42. Wash the precipitate completely with water containing a little hydrogen sulphide.

c) Dissolve the precipitate in 30 to 40 ml of warm hydrochloric acid (1 : 1) and wash the filter paper with a little water. Boil the solution to remove hydrogen sulphide (test the vapour with moist lead acetate paper). When hydrogen sulphide is completely removed cool, and neutralize with sodium hydroxide using methyl red indicator. Make it up to 500 ml in a volumetric flask.

d) Pipette 50 ml of the made up solution in a conical flask. Add 5 to 6 ml of ammonia buffer solution and 8 to 10 drops of eriochrome black T indicator. Titrate it with EDTA solution 0·01 M.

6.6.2.4 *Calculation*

$$\text{Zinc (as Zn), percent by mass} = \frac{V \times M_1}{M_2}$$

where

V = volume of EDTA solution (0·01 M) consumed;

M_1 = mass in mg of zinc per ml of EDTA solution (0·01 M); and

M_2 = mass in g of sample taken for test.

6.7 Determination of Cobalt

6.7.1 *Range* — For cobalt content up to 15 micrograms in the final aliquot.

6.7.2 *Outline of the Method* — In this method, after destruction of any organic matter, cobalt (with other trace metals) is extracted with hydrochloric acid; precipitation of iron and phosphate is prevented by the addition of citrate. Cobalt is then determined absorptiometrically as its magenta coloured complex with 2-nitroso-1-naphthol in toluene solution.

6.7.3 *Reagents*

6.7.3.1 *Sodium citrate solution* — 20 percent.

6.7.3.2 *Hydrochloric acid* — approximately 2 N.

6.7.3.3 *Sodium hydroxide solution* — approximately 2 N.

6.7.3.4 *Hydrogen peroxide* — 3 percent (10 volume).

6.7.3.5 *2-Nitroso-1-naphthol reagent* — Dissolve 1 g of 2-nitroso-1-naphthol in 100 ml of glacial acetic acid, and add 1 g of activated carbon. Shake the solution before use, and filter the required amount.

6.7.3.6 *Toluene* — (*see* IS : 537-1967*).

6.7.3.7 *Sodium sulphate* — anhydrous.

6.7.3.8 *Standard cobalt solution* — Dissolve 0·080 8 g $CoCl_2·6H_2O$ in water and dilute to 100 ml. Dilute 1 ml of this solution to 100 ml. 1 millilitre of this diluted solution contains 2 microgram of cobalt.

6.7.4 *Procedure* — Transfer a suitable aliquot (containing not more than 15 micrograms cobalt) of the acid solution prepared in **4.1** or **4.2** to a 100-ml beaker, add 10 ml of sodium citrate solution, dilute to approximately 50 ml with water, and adjust the *p*H to between 3 and 4 by the addition of hydrochloric acid or sodium hydroxide solution, using *p*H test paper (a precipitate of ferric hydroxide may form, but this can be dissolved by heating the solution), and cooling to room temperature. Add 10 ml of hydrogen peroxide and, after 5 minutes, 1 ml of 2-nitroso-1-naphthol reagent; heat to about 90°C, and then allow to stand for at least 30 minutes at room temperature. Transfer the solution to a 125-ml separating funnel, add exactly 10 ml of toluene, shake vigorously for 2 minutes, and discard the lower aqueous phase. To the toluene extract add 20 ml of hydrochloric acid, shake for 1 minute, and run off and discard the lower aqueous phase. Wash the toluene extract once with a few millilitres of water and discard the aqueous phase. Add 20 ml of sodium hydroxide solution, shake for 1 minute and again run off and discard the lower aqueous phase. Repeat the treatment of the toluene extract with a further 20 ml of sodium hydroxide solution, and then wash the toluene extract once with a few millilitres of water, then discard the aqueous phase. Finally run off the toluene phase through a little anhydrous sodium sulphate and a cotton wool plug into a glass stoppered tube.

Carry out a blank determination on all the reagents used, together with check standards containing 5 and 10 micrograms of cobalt.

Measure the optical densities of the test and blank solutions in a spectrophotometer at a wavelength of 367 nm, using 1 cm cell and toluene

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*Specification for toluene, pure nitration grade (*first revision*).

in the comparison cell. Read the number of micrograms of cobalt equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph (checked against the two standards) and so obtain the net measure of cobalt in the sample. Establish the calibration graph as follows:

> Measure appropriate amounts of standard cobalt solution, covering the range 0 to 50 micrograms of cobalt, into a series of 100-ml beakers, and proceed as in **6.7.4** commencing at 'dilute to approximately 50 ml............'. Measure the optical densities of the solutions and construct a graph relating the optical densities to the number of micrograms of cobalt.

6.8 Determination of chloride

6.8.1 *Reagents*

6.8.1.1 *Standards silver nitrate solution* — 0·1 N.

6.8.1.2 *Concentrated nitric acid*

6.8.1.3 *Ferric ammonium sulphate solution* — saturated in water and stabilized by addition of 50 ml of nitric acid.

6.8.1.4 *Standard ammonium thiocyanate solution* — 0·1 N.

6.8.2 *Procedure* — Dissolve about 0·2 g of the prepared sample, previously dried and accurately weighed, in about 40 ml of water. Add exactly 50 ml of standard silver nitrate solution and 5 ml of concentrated nitric acid. Add 0·5 ml of nitrobenzene and make up the volume of the mixture to exactly 100 ml with water. Take exactly 50 ml of the solution and add 2 ml of ferric ammonium sulphate solution. Titrate the excess of silver nitrate in this portion with standard ammonium thiocyanate solution to a blood red colour end point.

6.8.2.1 Carry out a blank test following the procedure given above but without using the material.

6.8.3 *Calculation*

6.8.3.1 Total chlorides (as Cl),

$$\text{percent by mass (on dry basis)} = \frac{7{\cdot}09\,(V_1 - V_2)\,N}{M} \quad \dots (X)$$

where

V_1 = volume in ml of standard ammonium thiocyanate solution used in the blank determination;

V_2 = volume in ml of standard ammonium thiocyanate solution used in the test with the material;

N = normality of standard ammonium thiocyanate solution; and

M = mass in g of the dried *prepared sample* taken for the test.

6.8.3.2 Express the ammoniacal nitrogen content, percent by mass, of the material determined as prescribed in Part 2 of this standard, in terms of chlorine (cl) as follows :

$$\text{Chloride equivalent of the ammoniacal nitrogen content, percent by mass} = 2{\cdot}531 \times A \qquad \ldots(Y)$$

where

A = the ammoniacal nitrogen content.

6.8.3.3 $$\text{Balanced chloride equivalent to sodium chloride (NaCl), percent by mass} = 1{\cdot}648 \times (X - Y)$$

where

$X - Y$ = balanced chlorides other than ammonium chlorides.

6.9 Determination of Lead

6.9.1 *Method A* — Colorimetric method using dithizone.

6.9.1.1 *Reagents*

a) *Standard lead solution* — Dissolve 0·40 g of lead nitrate [$Pb(NO_3)_2$] in water containing 2 or 3 ml of concentrated nitric acid and make up the volume to 1 000 ml with water. Transfer 10 ml of this solution to a volumetric flask, add 2 or 3 ml of concentrated nitric acid and dilute with water to 1 000 ml. One millilitre of this solution contains 2·5 mg of lead (as Pb). The diluted solution shall be freshly prepared.

b) *Dilute ammonium hydroxide* — 1 N, approximately.

c) *Reagent A* — Dissolve 25 g of triammonium citrate or 22 g of citric acid and 4 g of hydroxylamine hydrochloride in about 200 ml of water. Add dilute ammonium hydroxide to bring the *p*H to 8·5. Dilute the solution to 500 ml. Purify this solution by extracting with 15 ml portions of 0·01 percent dithizone solution until the final colour of the dithizone extract is green. Wash the aqueous remainder portion three times with 25 ml portions of chloroform and finally with one 25 ml portion of carbon tetrachloride.

d) *Dithizone solution* — Dissolve 0·01 g of dithizone in 100 ml of carbon tetrachloride, shaking intermittently for 1 hour. Allow to stand overnight and shake once again before using. This shall be kept in a cool and dark place. This gives a 0·01 percent solution, filter, if necessary. Dilute 10 ml of this solution to 100 ml with carbon tetrachloride in a 100 ml volumetric flask. This shall be prepared fresh before determination. This gives a solution of 0·001 percent.

NOTE 1 — Carbon tetrachloride used should be further purified. One litre of carbon tetrachloride is extracted with two portions of 25 ml dilute ammonium hydroxide and then kept over 100 g of activated carbon. Before use, it is decanted and distilled at about 80 °C over a little fresh lime.

NOTE 2 — Sometimes dithizone solid and its 0·01 percent solutions deteriorate on storage. The 0·01 percent solution should, therefore, be tested before further dilution, by shaking 2 ml of the solution with 5 ml of 1 percent ammonium hydroxide. If the organic layer is only faintly yellow under these conditions, the solution may be used. If it is deeply coloured it shall be discarded and fresh solution be prepared. The solution as well as the reagent should be stored in a refrigerator and exposure to sunlight should be avoided during analytical work. To increase the stability of 0·01 percent solution, it should be covered with a thin aqueous layer saturated with sulphur dioxide.

e) *Thymol blue indicator solution* — 1 percent (*m/v*) solution in rectified spirit.

6.9.1.2 *Apparatus* — Nessler cylinders (50 ml capacity).

6.9.1.3 *Preparation of the sample* — Weigh accurately 5 g of sample, dissolve in 100 ml of distilled water and add 1 ml of sulphuric acid. Filter the solution and make up to 250 ml with water in a volumetric flask. Take 50 ml of the above solution in a beaker, heat, pass hydrogen sulphide gas or add sodium sulphide solution, and ensure complete precipitation. Filter hot and keep the residue for analysis of lead.

6.9.1.4 *Procedure* — Take several aliquots of standard lead solution into a series of separating funnels, add 5 ml of the water and 100 ml of the reagent A. Then add 2 drops thymol blue indicator and bring the *p*H of the solution to 8·5 by addition of dilute ammonium hydroxide. Add 5 ml of the dithizone solution and shake well for about 10 seconds. Drain the organic layer into stoppered Nessler cylinders.

Dissolve the residue obtained in **6.9.1.3** in dilute nitric acid and dilute with water. Take a suitable aliquot of the solution and transfer to a separating funnel. Develop the colour as prescribed above. Drain the organic layer into a stoppered Nessler cylinder, add 1·0 ml of dilute ammonium hydroxide, and shake for about 10 seconds. Compare the colour developed with those of the standard solutions. Note the volume of the standard with which the colour of the solution matches.

NOTE — If the colour of the test solution is intermediate between two standard solutions, then the experiment is repeated by taking more number of standard solutions in that range and exact colour matching is arrived at.

6.9.1.5 *Calculation*

$$\text{Heavy metals (as Pb), percent by mass} = \frac{100 \times v \times f}{M}$$

where

V = volume in ml of standard lead solution matching with the test solution;

f = mass in g of heavy metals (as Pb) equivalent of 1 ml of standard lead solution; and

M = mass in g of the material in the aliquot taken for the test.

6.9.2 *Method 2* — Spectrophotometric method.

6.9.2.1 *Reagents*

a) *Standard lead solution* — Weigh 0·159 9 g of lead nitrate $Pb(NO_3)_2$ on a clean watch glass and transfer it to one litre flask through the funnel giving several washings to watch glass and funnel with glass distilled or demineralised water. Add 10 ml of concentrated distilled nitric acid and make volume up to the mark. Stopper the flask and shake the solution well. This is 100 ppm lead solution and should be stored in a clean bottle for further use. Dilute 10 ml of 100 ppm solution of lead to 100 ml with 1 percent nitric acid solution to get 10 ppm standard lead solution.

b) *Nitric acid solution (1 percent)* — Dilute 10 ml of concentrated distilled nitric acid to one litre with glass distilled water.

c) *Zinc sulphate solution (20 percent)* — Weigh 20 g of zinc sulphate ($ZnSO_4 \cdot 7H_2O$) and dilute to 100 ml with 1 percent nitric acid solution.

6.9.2.2 *Preparation of working standards* — Pipette the following volume of 10 ppm standard lead solution in 50 ml numbered volumetric flasks. Add 5 ml of 20 percent zinc solution to each flask and make the volume with 1 percent nitric acid solution.

Flask No.	*Volume of* 10 *ppm Lead Solution Taken* (ml)	*Volume of* 20 *Percent Zinc Sulphate Solution Add* (ml)	*Concentration of Lead After Making the Volume to* 50 ml (ppm)
1	0·0	5·0	0·0
2	2·0	5·0	0·4
3	4·0	5·0	0·8
4	6·0	5·0	1·2
5	8·0	5·0	1·6
6	10·0	5·0	2·0

Stopper the flasks and shake them well.

6.9.2.3 *Procedure* — Weigh 1 g of the material on a clean watch glass and transfer to 50 ml volumetric flask through the funnel giving washings with 1 percent nitric acid solution. Dissolve the material and make the volume with 1 percent nitric acid solution. Samples should be prepared in duplicate. Flame the standards and the samples absorption spectrophotometer at a wavelength of 217 mμ (lead line of the instrument).

6.9.2.4 *Calculation* — Prepare a standard curve of known concentrations of lead solution by plotting the absorbance values on *Y*-axis against their respective lead concentration on *X*-axis. Calculate the percentage lead in zinc fertiliser by multiplying lead concentration value calculated from standard curve by 0·005.

7. DETERMINATION OF SECONDARY ELEMENTS AND MICRONUTRIENTS BY ATOMIC ABSORPTION SPECTROPHOTOMETRIC METHOD

7.1 Apparatus

7.1.1 *Atomic Absorption Spectrophotometer* — Several commercial models are available. Since each design is somewhat different, with varying requirements of light source, burner flow rate, and detector sensitivity, only general outline of operating parameter is given in Table 1.

TABLE 1 OPERATING PARAMETERS

Sl No.	Elements	Wave-length A°	Flame	Range µg/ml	Remarks
(1)	(2)	(3)	(4)	(5)	(6)
i)	Ca	4 227	Rich Air-C_2H_2	2 to 20	1 percent lanthanum 1 percent hydrochloric acid
		4 227	Rich N_2O-C_2H_2	2 to 20	Requires special burner
ii)	Cu	3 247	Air-C_2H_2	2 to 20	—
iii)	Fe	2 483	Rich Air-C_2H_2	2 to 20	—
iv)	Mg	2 852	Rich Air-C_2H_2	0·2 to 2	May need lanthanum if high concentration of aluminium or phosphorus are present
v)	Mn	2 795	Air-C_2H_2	2 to 20	—
vi)	Zn	2 138	Air-C_2H_2	0·5 to 5	—
vii)	Mo	3 133	N_2O-C_2H_2	10 to 100	Require 2 percent ammonium chloride solution and 2 000µg/ml aluminium in sample solution special burner needed
		3 133	Air-Rich C_2H_2		Three slot burner to be used

7.2 Reagents

7.2.1 *Standard Solution* — Do not use less than 2 ml pipettes and not less than 25-ml volumetric flasks. Automatic dilution apparatus may be used. Prepare standard solutions in 0 to 20 μg range fresh daily.

7.2.2 *Calcium Solutions*

7.2.2.1 *Stock solution* — (25 μg calcium per ml) — Dissolve 1·249 g of calcium carbonate in minimum amount of hydrochloric acid (3 N). Dilute it to 1 litre. Then dilute 50 ml of this solution to 1 litre.

7.2.2.2 *Working standard solution* — 0, 5, 10, 15 and 20 g calcium per ml containing 1 percent lanthanum to 25-ml volumetric flasks, add 0, 5, 10, 15 and 20 ml of calcium stock solution. Add 5 ml of lanthanum stock solution and dilute it to 25 ml.

7.2.3 *Copper Stock Solution* — (1 000 μg copper per ml) — Dissolve 1·000 g of pure copper metal in minimum amount of nitric acid and add 5 ml of hydrochloric acid. Evaporate almost to dryness and dilute to 1 litre with hydrochloric acid 0·1 N.

7.2.4 *Iron Stock Solution* — (1 000 μg iron per ml) — Dissolve 1·000 g of pure iron wire in about 30 ml of hydrochloric acid (6 N) with boiling. Dilute to 1 litre.

7.2.5 *Lanthanum Stock Solution* — (50 μg of lanthanum per litre) — Dissolve 58-65 g of lanthanum oxide in 250 ml of hydrochloric acid, adding acid slowly. Dilute it to 1 litre.

7.2.6 *Magnesium Stock Solution* — (1 000 μg mangesium per ml) — Place 1·000 g pure magnesium metal in 50 ml of water and slowly add 10 ml of dilute hydrochloric acid. Dilute it to 1 litre.

7.2.7 *Manganese Stock Solution* — (1 000 μg manganese per ml) — Dissolve 1·582 g of manganese oxide in about 30 ml of hydrochloric acid (6 N). Boil and dilute it to 1 litre.

7.2.8 *Zinc Stock Solution* — (1 000 g zinc per ml) — Dissolve 1·000 g of pure zinc metal in about 10 ml of hydrochloric acid (6 N). Dilute it to 1 litre.

7.2.9 *Molybdenum Standard Solution* — (1 000 μg per ml) — Dissolve 1·840 g of ammonium molybdate tetrahydrate (analytical reagent grade) in water and dilute it to 1 litre.

7.2.10 Dilute aliquots of solution prepared in **7.2.3** to **7.2.9** with hydrochloric acid (0·5 N) to make four or more standard solutions of each element within the range of determination.

7.3 Preparation of Sample Solutions

7.3.1 *For Inorganic Materials and Mixed Fertilizers* — Dissolve 1·0 g of the well ground sample in 10 ml of hydrochloric acid in 100-ml beaker. Boil and evaporate the solution nearly to dryness on hot plate. *Do not bake the residue.*

Re-dissolve the residue in 20 ml of hydrochloric acid (2 N) , boiling gently if necessary. Filter through fast paper into 100-ml volumetric flask, and wash the paper and residue thoroughly with water. Measure the absorption of the solution directly, or dilute with hydrochloric acid (0·5 N) to obtain solutions within ranges of instrument. If calcium is to be determined, add enough *lanthanum stock solution* to make final dilution of 1 percent lanthanum (5 ml lanthanum to 25-ml flask, 20-ml lanthanum to 100-ml flask).

7.3.2 *For Fertilizers Containing Organic Matter (Tankage, Corncobs, Cotton-seed Hulls, etc)* — Place 1·0 g of the sample in 100 ml beaker. Char on hot plate and ignited for 1 hour at 500°C with muffle door propped open to allow free access of air. Break up cake with stirring rod and dissolve in 10 ml of hydrochoric acid as in **7.3.1**.

7.3.3 *For Fertilizer Containing Fritted Trace Elements* — Dissolve less than or equal to 1·0 g of the well ground sample in 5 ml of perchloric acid and 5 ml hydroflouric acid. Boil and evaporate to dense perchloric acid fumes. Dilute carefully with water, filter and proceed as in **7.3.1.**

Alternatively dissolve the sample in 10-ml of hydrochloric acid, 5 ml of hydrofluoric acid and 10 ml of methyl alcohol. Evaporate to dryness. Add 5-ml of hydrochloric acid and evaporate. Repeat hydrochloric acid addition and evaporation. Dissolve residue as in **7.3.1.** (Normally platinum ware should be used; borosilicate or other glassware may be used if sodium potassium, calcium and iron are not to be determined.)

7.3.4 *For Manganese*

7.3.4.1 *Acid soluble for both Mn^{+2} and Mn^{+4}* — Follow the appropriate procedure prescribed as in **7.3.1**, **7.3.2** and **7.3.3** and then proceed as in **7.3.4** using standard solutions prepared in **7.2.7**.

7.3.4.2 *Acid soluble manganese (applicable to Mn^{+2})* — Place 1g of the sample in 200 ml conical flask. Add 10 ml of sulphuric acid and 30 ml of nitric acid. Heat gently until brown fumes diminish, then boil for 30 minutes. If organic matter is not destroyed, cool add 5 ml of nitric acid and boil. Repeat this process until no organic matter remains and boil until white fumes appears. Cool dilute to 200 ml in volumetric flask, mix, and let it stand to allow precipitation of calcium sulphate. Proceed as in **7.4** using standard solution prepared as in **7.2.7** and **7.2.10** substituting 0·5 N H_2SO_4 for 0·5 N HCl in **7.2.10**.

7.3.5 *For Iron and Zinc*

7.3.5.1 *Aqueous extraction* — Place 1·00 g sample in 250 ml beaker, add 75 ml of water and boil for 30 minutes. Filter into 100 ml volumetric flask, washing filter paper with water. Dilute to volume and redilute if necessary.

7.3.5.2 *Chelation extraction* — Place 1·00 g sample in 250 ml beaker and add 5 cm (2") magnetic stirrer bar and 100 ml of 2·5 percent EDTA solution. Stir exactly for 5 minutes and filter through Whatman No. 40 paper or equivalent, if filtrate is cloudy, refilter immediately through fine paper (Whatman No. 5 or equivalent). Redilute, if necessary, with hydrochloric acid (0·5 N).

7.4 Determination

7.4.1 Set up instrument as in Table 1. Read four or more standard solutions prepared within analytical range before and after each group of 6 to 12 samples. Flush burner with water between samples and re-establish '0' absorption point each time. Prepare calibration curve from average of each standard before and after sample group. Read concentration of samples from plot of absorption against μg/ml.

NOTE — Phosphorus interferes in calcium determination and may interfere in magnesium determination with air-C_2H_2 burners. Eliminate interference by adding lanthanum stock solution to standard and sample solutions so that final dilution contain 1 percent lanthanum. Phosphorus does not interfere with calcium determination when N_2O – C_2H_2 flame is used.

7.5 Calculation

$$\text{Percent element} = (\mu g/ml) \times (F/\text{sample mass}) \times 10^{-4}$$

where

F = ml original dilution × ml final dilution/ml aliquot; if original 100 ml volume is diluted.

APPENDIX A

(*Clause* 0.4)

CORRESPONDING TEST METHODS IN IS : 6092 (Part 5) - 1971 AND IS : 6092 (Part 5) - 1985 (*first revision*)

Sl No.	*Test Method*	*Clause Ref in* IS : 6092 (Part 5)- 1971	*Clause Ref in* IS : 6092 (Part 5)- 1985 (*first revision*)	*Remarks*
(1)	(2)	(3)	(4)	(5)
i)	Preparation of sample solution	5	4	
ii)	Determination of boron	6	6.1	
iii)	Determination of chromium	7	—	Method of test for chromium has been deleted in the first revision as this element is an impurity and not a micronutrient
iv)	Determination of cobalt	8	6.7	
v)	Determination of copper	9	6.2	
vi)	Determination of fluorine	10	—	Method of test for fluorine has been deleted in the first revision as this element is an impurity and not a micronutrient
vii)	Determination of iodine	11	—	Method of test for iodine has been deleted in the first revision as this element is an impurity and not a micronutrient
viii)	Determination of iron	12	6.3	

Sl No.	*Test Method*	*Clause Ref in* IS : 6092 (Part 5)-1971	*Clause Ref in* IS : 6092 (Part 5)-1985 (*first revision*)	*Remarks*
ix)	Determination of magnesium	13	5.2	
x)	Determination of manganese	14	6.4	
xi)	Determination of molybdenum	15	6.5	
xii)	Determination of nickel	16	—	Method of test for nickel has been deleted in the first revision as this element is an impurity and not a micronutrient
xiii)	Determination of zinc	17	6.6	
xiv)	Determination of calcium	—	5.1	These methods of test have been incorporated in the first revision
xv)	Determination of sulphur	—	5.3	
xvi)	Determination of chloride	—	6.8	
xvii)	Determination of lead	—	6.9	

(*Continued from page* 2)

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IS 6092 (Part 5) : 1985 METHODS OF SAMPLING AND TEST FOR FERTILIZERS

Part 5 DETERMINATION OF SECONDARY ELEMENTS AND MICRONUTRIENTS

(First Revision)

(*Page* 18, *clause* **6.1.3.6**) — Add the following new matter:

'**6.1.4** *Method D (Atomic Absorption Spectrometer Method)*

6.1.4.1 *Reagents*

Standard Boron Solution — Weigh 44.095 g of sodium tetraborate decahydrate ($Na_2B_4O_7 10H_2O$) — AR grade on clean watch glass and transfer it to one litre volumetric flask through the funnel giving several washings to watch glass and funnel with glass distilled water. Make the volume up to the mark. Stopper the flask and shake the solution well. This is 5000 ppm boron solution hereinafter called standard A. This solution should be stored in clean bottle for further use.

Preparation of Working Standards — Pipette the following volume of standard A in 50 ml numbered volumetric flasks and make the volume with water.

Flask No.	*Volume of standard A taken (ml)*	*Concentration of boron after making volume to 50 ml (ppm)*
1.	0.0	0.0
2.	4.0	400
3.	6.0	600
4.	8.0	800
5.	10.0	1 000
6.	12.0	1 200
7.	14.0	1 400
8.	16.0	1 600

6.1.4.2 *Procedure*

Preparation of sample solution — **Weigh 2.500 g of the material on a clean watch glass and transfer it quantitatively in a 250-ml volumetric flask through the funnel giving repeated washing with water. Make up the volume, stopper the flask and shake well.**

Flamming the solutions — Flame the standards and the sample solution on atomic absorption spectrophotometer at a wavelength of 249.6 nm using nitrous oxide-acetylene flame.

Calculations : Prepare a standard curve of known concentrations of boron solutions by plotting the absorbance value on *Y*-axis against their respective boron concentration on *X*-axis. Calculate the percentage boron in the given sample by multiplying boron concentration value obtained from standard curve by 0.01.

Example:

Mass of the fertilizer sample = 2.50 g
Volume made = 250 ml
Dilution factor $\frac{250}{2.5} = 100$

Reading of sample from atomic absorption = Y

Corresponding concentration value of boron from standard curve against Y absorbance — X ppm

percentage of boron in the sample — $0.01\,X$

(PCD 20)

Reprography Unit, BIS, New Delhi, India